International Journal of Infectious Diseases (2006) **10**, 93—102


http://intl.elsevierhealth.com/journals/ijid

REVIEW

# Vaccination strategies to prevent tuberculosis in the new millennium: from BCG to new vaccine candidates

**Carlos Franco-Paredes** [a,b,*], **Nadine Rouphael** [b], **Carlos del Rio** [b], **José Ignacio Santos-Preciado** [a]

[a] *Hospital Infantil de México Federico Gómez, Mexico*
[b] *Division of Infectious Diseases, Emory University School of Medicine, 69 Jesse Hill Jr. Drive, Atlanta GA 30303, USA*

Received 25 March 2005; received in revised form 23 June 2005; accepted 30 June 2005
**Corresponding Editor:** Raymond Smego, Sohar, Oman

**KEYWORDS**
Bacille Calmette—Guérin;
Tuberculosis;
Vaccines

**Summary** Current global control efforts targeting tuberculosis (TB) include the treatment of latent TB infection, case detection and treatment with directly observed therapy short-course (DOTS), and BCG (bacille Calmette—Guérin) vaccination. However, BCG has been found to decrease only childhood TB morbidity and mortality but has a very limited effect in the transmission dynamics of the infection. These limitations of BCG are the driving force for the development of new TB vaccines. New TB vaccine candidates have entered clinical evaluation and many more are in the pipeline to undergo clinical testing. New vaccine candidates may offer better protection than that afforded by currently available BCG vaccines. Furthermore, combined vaccination schedules against TB seem to be a promising strategy in the new millennium.


## Introduction

Infectious diseases remain a major cause of death worldwide and also represent an incalculable source of human misery. More than 95% of these deaths occur in the developing world.[1,2] The three major infectious disease killers are HIV/AIDS, tuberculosis (TB), and malaria. In 1993 the World Health Organization (WHO) declared TB a global emergency due to its medical, social, and economic consequences.[3]

Currently, global control efforts targeting TB include the treatment of latent TB infection (LTBI), case detection and treatment with directly observed therapy, and BCG vaccination.[3] However, altogether these strategies have had a limited impact in reducing the global burden of TB despite

* Corresponding author. Tel.: +1 404 616 3600; fax: +1 404 880 9305.
*E-mail addresses:* cfranco@sph.emory.edu, carlos_franco@emoryhealthcare.org (C. Franco-Paredes).

1201-9712/$32.00 
doi:10.1016/j.ijid.2005.06.002

growing adherence to the WHO recommendations on the use of directly observed therapy short-course (DOTS) in many countries and the widespread use of BCG vaccination in many regions of the world.[4] Although modern chemotherapy to treat TB disease is highly effective, the effect that the concomitant HIV pandemic has had on TB, the length of treatment and the requirement for multiple drug combinations have limited the impact that thus far these global efforts to control TB have had.[5]

The immunological events elicited by *Mycobacterium tuberculosis* infection are critical to the clinical expression of the disease and to evaluate vaccine efficacy.[6] Data from clinical trials and observational studies have shown widely disparate results with BCG vaccination.[7] In some studies BCG use has shown significant protection and in others offered no benefit.[8–24] Another downside to BCG vaccination has been concern regarding its safety.[25–27] As a result, there is an urgent global public health need to develop newer efficacious and safer TB vaccines that may have an impact in both preventing TB infection and, in previously infected populations, halting the progression to TB disease.[28–32]

The future role of current BCG vaccination policies is unclear in the setting of the possible introduction of newer TB vaccines. Therefore, the objective of this study is to review the published medical literature on the topic and objectively analyze the possible impact of new TB vaccine candidates and compare this information to the known biomedical and public health impact of currently used BCG vaccines.

## The rise and fall of BCG

The BCG vaccine was prepared at the Institut Pasteur in Lille, France in 1921 by Calmette and Guérin and consists of a live-attenuated strain of *Mycobacterium bovis*, a closely related subspecies of *M. tuberculosis*. While working with culture medium to decrease bacterial clumping of *M. bovis* Calmette and Guérin found that when beef bile was added the virulence of the *M. bovis* strain was reduced. It was after 231 serial passages between 1908 and1924, that the vaccine offered protection in animal models.[7,33–37] Recent genotypic analyses suggest that the RD1 region of the *M. tuberculosis* genome, which codes for nine proteins, is missing in BCG.[33] Deletion of this region has been considered to be a key event leading to the attenuation features of the mycobacterial strain.[38] The original BCG strain was maintained by serial passage at the Institut Pasteur. Before this original strain was lost, it was distributed to dozens of laboratories in many countries. Each laboratory produced its own BCG and has maintained it by serial passages.[3,4,34]

Clinical use of BCG vaccines started during the 1920s.[30] Its use was supported by the results of early trials conducted by Heimbeck among nursing students in Norway which demonstrated that nursing students vaccinated with BCG had lower rates of active TB when compared to those who did not received the vaccine.[39] Additional evidence came from the first formal trials organized among North American Indians in the 1930s.[17–19,21] From 1929 to 1930, many orally vaccinated children in Lubeck, Germany died of TB because the oral BCG preparation was contaminated with a virulent tubercle strain.[40] By the 1940s several other clinical studies had confirmed the evidence of protection of BCG vaccines.[17–20] Subsequently, and based on the recognition that tuberculosis rates increased in the aftermath of World War II, vaccination was widely applied by various international health organizations.[3,7,30] In the 1960s WHO developed recommendations for routine BCG vaccination.[30] BCG was incorporated into the Expanded Program on Immunizations (EPI) infant vaccination schedule in 1974.[3,30] Today BCG vaccines are among the most widely used in the world and have been administered to over 3 billion individuals in over 80 years.[3,30] Currently available BCG vaccines are produced by more than 40 manufacturers around the world.

While BCG is considered one of the oldest vaccines currently used throughout the world, policies for its use differ between countries, and there is a long-standing history of controversy concerning the efficacy of BCG and its overall impact on TB.[3–5,30] BCG vaccination policies can be divided into four different categories: (1) those that recommend BCG at birth or first contact with the healthcare system; (2) those that recommend BCG only once in childhood; (3) those that recommend repeated BCG boosters; and (4) those countries where BCG is not recommended.[3,30] Almost all countries of the world, with the exception of a few industrialized countries such as the United States and the Netherlands have routinely recommended BCG vaccination. The reasons for these variations in BCG vaccination policies reflect medical and public health strategies of the countries involved. Currently, the prevailing policy is only one dose at birth as recommended by the WHO.[3,4,30] The International Union against Tuberculosis and Lung Disease (IUATLD) has developed criteria for a country to shift from routine BCG vaccination to selective vaccination of only high risk groups, an approach recently adopted in the United Kingdom.[3,30]

## Efficacy of BCG

When evaluating BCG vaccine efficacy it is fundamental to consider the protective effect of BCG separately in children and adults. Different clinical trials evaluating the efficacy of BCG have demonstrated contradictory findings, varying from a BCG efficacy rate in preventing adult pulmonary TB that ranges from 0% in South India to 80% in the UK.[7–24] Vaccination of children with BCG after birth, as recommended by WHO, has not been shown to prevent the majority of infectious pulmonary TB cases among adults. In addition, it has never been clearly established that using BCG boosters may enhance or maintain protection against TB. When the results from well-designed clinical controlled trials are combined, with both children and older individuals included, an efficacy of approximately 50% has been identified.[15] The most recent meta-analysis of previously published data reviewed 1264 titles or abstracts and 70 selected studies, but only 26 studies met the inclusion criteria. This analysis revealed similar results to those above, with BCG vaccination reducing the risk of developing TB by an average of 50%.[11]

As controversial as has been the protective effect of BCG on adult cases of pulmonary TB disease, there is clear evidence that BCG provides consistent and appreciable protection against tuberculous meningitis and disseminated disease in children.[8,9] A meta-analysis of five randomized controlled trials and eight case-control studies demonstrated an average protection of 86% (95% CI 65—95%) for controlled trials and 75%, (95% CI 61—84%) for case-control studies.[9] However, the evidence in terms of protection against pulmonary TB disease during childhood is also controversial.[8,9,30] When analyzing trials that included mycobacterial naïve cohorts of newborns and infants with follow-up periods between 7 and 19 years, an efficacy rate of 73% is found.[18–20,41]

Various hypotheses have emerged to explain the different results of various clinical trials with BCG.[3,7,22,23,30,41,42] One hypothesis attributes the differences to the variation among strains of BCG and another to previous exposure to various environmental mycobacteria.[23,38,43–45] The Chingleput trial in South India, conducted in 1968, evaluated two BCG strains, Paris/Pasteur versus Danish/Copenhagen in an area with high tuberculin positive prevalence attributed to environmental mycobacteria.[3,22] This area also had high rates of TB. The results of this trial showed that neither vaccine was protective for pulmonary TB. These results led to a series of other clinical studies aimed at evaluating BCG use in different populations.

Several authors have evaluated whether the differences in various biological properties of BCG vaccines could explain the different observed degrees of protection.[23,33,43] Exposure to environmental mycobacteria may explain some of the observed variation in vaccine efficacy, perhaps by a crossed-antigenicity effect evoking a hampered BCG response when individuals have been previously exposed to environmental mycobacteria.[23]

A relevant aspect of TB pathogenesis that may additionally impact BCG immunization efficacy is exogenous reinfection. The mechanism of action of the vaccine to induce protection is based on its ability to limit bacillemia associated with primary pulmonary infection.[6] This hematogenous spread is believed to be a central component of the pathogenesis of TB.[6,7,30,34,37] The contribution of exogenous reinfection in decreasing immunization efficacy has been demonstrated, although the extent of this contribution is not known.[46,47] These events are particularly relevant since exogenous reinfection to the lung apex as a second pathway to cavitary disease may be indistinguishable from primary hematogenous seeding. Thus, these reinfection events may impact vaccine efficacy by bypassing hematogenous dissemination blocked by immunization, leading to continuing TB transmission dynamics.

Finally, immunogenetic factors at a population genetic level have been suggested as further possible factors in BCG biological behavior, associated with cellular immune mechanisms such as specific HLA-haplotypes, interferon-$\gamma$ (INF-$\gamma$) polymorphisms and to the NRAMP gene influencing susceptibility to TB and other mycobacterial infections.[3,5,30,41,47]

## Cross-protection of BCG against other mycobacterial infections

The efficacy of BCG against leprosy and other mycobacterial infections is considered another important benefit of using this vaccine. More than four clinical trials and more than 10 observational studies have all shown protection ranging from 20 to 80%.[48–53] There is more evidence of protection against lepromatous leprosy than against tuberculoid leprosy and therefore its use has significant public health implications, given the fact that the polar lepromatous forms are considered to be responsible for most transmission of leprosy in developing countries. While there is no convincing evidence that the use of booster doses of BCG provides additional TB protection, there is enough evidence to support the use of BCG boosters to protect against leprosy.[50]

An additional advantage of BCG vaccination is protection against lymphadenitis caused by non-tuberculous mycobacteria and against Buruli ulcer caused by *Mycobacterium ulcerans*.[54] This evidence is based upon observations where increases in non-tuberculous mycobacterial lymphadenitis were identified in child cohorts after universal BCG vaccination policies were discontinued in some European countries.[43–45,55]

## Adverse events of BCG vaccination

Overall, the side-effects associated with BCG are tolerable and historically these adverse events have varied with the use of different BCG strains.[3,30,56,57]

Nowadays, BCG is the only commonly used vaccine that induces ulceration at the inoculation site. The probability of leaving a scar is lower when the vaccine is administered during early infancy.[3,30] Inoculation of BCG results in a local inflammatory response that usually persists for several months. The expected reaction is the formation of a cold abscess at the site of injection that turns into a papule after 2–3 weeks.[3,41] The lesion reaches maximum size at about 6 weeks, when the overlying skin becomes thin and shiny and frequently ulcerates. This lesion usually heals at around 10 weeks. Side effects are common, including local reactions such as erythema, pain, and swelling in the administration site.[3,30,41] Local abscesses have been reported in 2% of vaccinees secondary to inadequate administration technique when the vaccine is inoculated into the subcutaneous space instead of being intradermal. In addition, lymphadenitis occurs in approximately 1% and osteitis in 0.04% of vaccine recipients and has been reported mostly in Scandinavia and Eastern Europe.[25–27,41] Other reports have suggested that osteitis occurs at a rate of 3–7 per 100 000 vaccinations with predilection for the metaphyses of the long bones, lower extremities, and ribs. However, serious adverse reactions are rare, with an incidence of $<1$ case in 1 million.[3,24–26] Disseminated BCG infection occurs mostly in immunocompromised infants with such conditions as severe combined immunodeficiency, and among children with HIV infection.[24,25]

## To BCG or not to BCG?

While it has become clear that the use of BCG vaccines in millions of people in many areas of the world has not impacted the epidemiology of TB on a global scale, it is widely accepted that millions of cases of meningeal and disseminated tuberculosis in children have been prevented by its widespread use in regions with high incidence rates of TB. The reasons why BCG has failed to reduce the global burden of TB are probably multifactorial. In addition, the impact of BCG vaccines is difficult to demonstrate since the impact of this vaccine is not as readily apparent as with other routinely administered childhood vaccinations. Furthermore, the main burden of TB is carried by adults with pulmonary disease, in whom BCG has consistently been shown to have no significant protective effect.[58] However, recent long-term efficacy data of BCG use has been reported after a follow-up period of more than 60 years with surviving members of a cohort of patients that participated in a clinical trial between 1935 and 1938 among American Indians and Alaskan natives.[10,29,59] In this report BCG vaccination demonstrated an efficacy of 52%, with incidence rates of 66 and 138 per 100 000 per year in the vaccinated and unvaccinated groups, respectively. In this trial, BCG vaccine efficacy persisted for more than 50 years, suggesting that a single dose of an effective BCG vaccine may have long-lasting protection.[29,59] The results of this trial have brought back to the surface the controversy of longstanding protection of BCG vaccination occurring during childhood and persisting during adult life with a single dose of BCG.

Many regions of the world continue to have elevated rates of TB disease that preclude the use of preventive chemotherapy as the sole component of TB prevention. It is in these regions that BCG vaccination continues to be an important component of the control strategies to decrease the complications of TB disease. In this regard, our experience in Mexico during the period 1986 to 2000 at the National Immunization Council is illustrative. In 1992, the WHO Global Health Report noted that Mexico had 37 626 cases of active TB. Despite this number of TB cases, we have been able to demonstrate the benefits of BCG vaccination as part of the Expanded Program on Immunizations. In Mexico, most children receive a BCG dose after birth or at their first encounter with the health system. During this period we identified a decreased rate of TB meningitis in children that coincides with the amount of routinely administered BCG doses (Figure 1). Over the years, we have witnessed a reduction in the rates as well in the absolute number of TB meningitis cases in children with minimal adverse events registered at the national vaccine adverse events system.

A delay in vaccination after birth may fail to prevent cases of childhood tuberculosis if a family member who has the disease is present within the household. Most transmission to children occurs

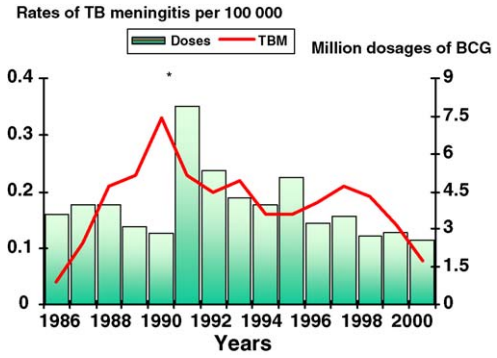


**Figure 1** Rates of tuberculous meningitis with regards to the number of BCG vaccines administered in Mexico during the period 1986—2000. *During the period 1989—1991, there was a significant increase in the rates of TB meningitis due to a decrease in vaccination coverage. Similar trends were observed between 1993 and 1994, and 1996 and 1997. Source: National Immunization Council, Mexico.

before the adult source case is identified, and given the short incubation period for meningeal and disseminated TB, implies that the time to medically intervene is very limited.[58] Only effective BCG vaccination of children reduces the development of meningeal disease. Therefore, regardless of the absence of an effective reduction in the transmission rates of TB, from a public health perspective the benefits of BCG vaccination of children in areas with high rates of TB clearly outweigh the risks.

## New TB vaccine candidates

Advances in tuberculosis research over the last few years have been driven by genomics, proteomics, and advances in immunology.[60,61] With the sequencing of the genome of *M. tuberculosis* much insight has been gained into the pathogenesis of the infection and lines of molecular research to promote the development of new TB vaccines have been established.[62] These approaches have refined the selection of potential candidate antigens of *M. tuberculosis*. With one third of the human population worldwide infected with *M. tuberculosis* a post-exposure or post-infection vaccine should be considered a major target in the TB vaccine development efforts.[14,61,62] Overall, it has been estimated that even a highly effective vaccine (50 to 90%) will only reduce the number of TB cases by one third. However, this partial benefit may translate into reduced TB mortality rates and decreased emergence of multidrug-resistant strains.[14]

The development of vaccines is focused on the induction of protection by generating an immune response similar to natural infection without causing disease, so that re-infection does not occur.[37,41] In the case of TB, the associated high rates of reactivation and re-infection discloses the inefficient immunity evoked by natural infection. Newer approaches are thus required, to rationally design new TB vaccines in order to evoke a superior immune response than that induced by natural TB infection.[5]

The primary goal of TB vaccines is to induce memory pools of Th1-type cells that are considered the main effectors in acquiring immunity against *M. tuberculosis*.[5,13,62] Both CD4+ and CD8+ T-cells play a major role in optimal protection against TB. Historically, the lack of reliable laboratory or serologic markers for immunity to mycobacteria has negatively impacted on efforts to determine how BCG affects the host and what level of protection it provides.[5] While there is extensive evidence that INF-$\gamma$ secreting T-cells are an essential component of mycobacterial immunity, it remains to be determined whether the ability of a vaccine to prime such cells is correlated with its protective efficacy.[5,60,61] To answer this issue, novel whole blood intracellular cytokine detection assays have been developed to determine specific T-cell responses.[63—66] These assays have been shown to be specific and sensitive for detecting mycobacteria-specific immunity following BCG vaccination performed in rural field studies.[63—65] A whole-blood luminescence model assay has also been developed to detect changes in the cellular immune responses to mycobacteria induced by BCG vaccination. This optimized assay, which measures immune responses to mycobacteria by the use of reporter gene-tagged BCG (BCG lux), has been determined to be a useful tool in evaluating the immunogenicity of newly developed vaccine candidates prior to large field trials in developing countries to assess vaccine efficacy.[64]

Vaccines under development are being designed using either live attenuated mycobacteria, or by attempting to deliver selected mycobacterial genes or gene products. Biological strategies currently under evaluation include: use of prime-boost approaches, subunit vaccines, DNA vaccines, modifying currently available BCG vaccines, or attenuating *M. tuberculosis* strains.[41,60—62]

Tuberculosis vaccine research has shifted from developing new vaccine candidates, to selecting the best vaccines for clinical evaluation.[13] Approximately 268 TB vaccine candidates have been analyzed for their ability to protect against *M. tuberculosis* infection in animal models. Many of these successful candidates identified in experimen-

tal animal models have begun to be tested in phase 1 trials in humans.[5,41,62]

## Prime-boost vaccines

The use of vaccination to induce a protective humoral immune response is a widely established principle to protect against many infectious pathogens.[67] In general, repeated vaccination with the same vaccine results in higher levels of antibodies. The prime-boost approach is based on the concept of successive administration of the same mycobacterial antigen. Homologous boosting refers to the antigens being delivered by the same delivery system with each subsequent dose.[28,67,68] Heterologous boosting consists of the successive administration of the same mycobacterial antigens expressed by different vaccine vectors, such as intramuscular administration of naked DNA expressing the antigen followed by intradermal inoculation with a modified *Vaccinia* virus expressing the same mycobacterial antigen.[67]

Vaccination against intracellular pathogens such as *M. tuberculosis* needs to induce strong cellular immune responses.[5] In this regard, heterologous-boosting approaches using mycobacterial antigen have been shown to improve the induction of cell-mediated immunogenicity of vaccine candidates.[28,67] In the case of *M. tuberculosis* heterologous prime-boost immunization strategies have been shown to induce higher levels of both CD4+ and CD8+ T-lymphocytes than homologous boosting. Recombinant pox-viruses have been used efficiently to induce this particular type of response. Using BCG as the priming immunization by this prime-boost approach seems to be a practical solution that provides for the beneficial effects of BCG in children to be maintained into adulthood.[67,68]

The results of some of these phase 1 trials with the MVA subunit vaccine have already been published.[68] The initial trial focused on using the prominent mycobacterial antigen 85A delivered as a recombinant smallpox vaccine (modified vaccinia Ankara, MVA); the 85A antigen is conserved among all mycobacterial species and is present in all strains of BCG.[28,68] It has been suggested that in the case of TB, by incorporating BCG into such a prime-boost schedule, the protective effects of BCG might be retained.[68] The trial was conducted in Oxford, UK, where school children no longer routinely receive BCG, and demonstrated a safety profile and adequate immunogenicity. Interestingly, individuals who had previously received BCG and received the MVA85A vaccine as a booster developed stronger immune responses that at 24 weeks were 5 to 30 times higher than those in BCG-naïve volunteers.[68]

## Subunit vaccines

Subunit vaccines deliver specific mycobacterial immunogenic antigens that have the ability to induce a protective effect.[41] These antigens are delivered in one or more forms: protein or peptides (sometimes given in a combination of various proteins), DNA, or live vectors.[41,60,62] The subunit approach would require only one or more antigens intended to induce specific subpopulations of CD4+ T-cells and perhaps of some CD8+ T-cells, whereas a whole bacterial vaccine attempts to stimulate many T-cell subpopulations at the same time.[41]

Modifying current BCG vaccines by restoring lost genes or increasing the expression of remaining antigen-producing genes might become an ideal strategy. The most studied gene in the RD1 region is responsible for encoding ESAT-6, a protein of unknown function that stimulates a strong T-cell response.[5,62] The immune response to ESAT-6 has been shown to be an element of protective immunity, and subunit vaccines that induce ESAT-6 reactive T-cells provide partial protection against tuberculosis in animal models.[5,41]

Pym et al.[38] have demonstrated that several genes in the RD1 region are required for secretion of ESAT-6, which was also shown to be an essential component in the induction of an optimal T-cell response. They also observed that immunization with a recombinant BCG strain carrying the RD1 region improves protection against aerosol challenge with *M. tuberculosis* in animal models. An interesting observation in this animal model was that it decreased the amount of the bacterial load identified in the spleen, which suggests an enhanced ability to restrict the hematogenous spread of *M. tuberculosis*.[38,69] In addition, combination of immunodominant secreted antigens such as ESAT-6 and Ag85B have shown protection in animal models.[41,69]

## DNA vaccines

DNA that encodes mycobacterial antigens can be inserted together with a suitable promoter in a bacterial plasmid.[70] The intramuscular injection of this complex induces an immune response to the mycobacterial antigen encoded by the DNA. In this manner, the response is strong, since bacterial DNA, unlike vertebrate DNA, is recognized as foreign by vertebrates because of its high content of

unmethylated CpG motifs.[41,70,71] Subsequently, these motifs are recognized by toll-like receptors that are expressed by different cells of the innate immune system, and therefore stimulate the production, activation, and maturation of dendritic cells. These cells, in turn, preferentially induce a Th1 CD4+ response.[62] Some plasmids may be administered adsorbed to tiny beads and blasted through the skin and enter Langerhans' cells directly, subsequently activating the same Th1 cascade that controls many intracellular bacterial infections.[41,60,70] DNA vaccination strategies with plasmid encoding proteins Ag85 and Hsp65 are now in the pipeline. Hsp65 is a highly conserved antigen from *Mycobacterium leprae* that has shown protection equal to BCG in the mouse model.[72,73] However, studies in non-human primates and clinical phase 1 studies in humans, suggest that the strong cellular immune responses seen in small animals are often not found when these vaccines are tested in larger species.[41,71–77]

## Attenuated vaccines

Attenuated BCG or *M. tuberculosis* whole cell vaccines have also been evaluated as possible vaccine candidates.[41,60–62] The goal of this approach is to inactivate particular groups of genes associated to virulence of these strains while maintaining the ability to induce protective immune responses. Some of the strategies to obtain these effects using whole cell live vaccines include: modifying their amino acid biosynthetic pathways (BCG or *M. tuberculosis*); nutritionally deficient strains (BCG or *M. tuberculosis*); diminished superoxide dismutase activity (BCG) or overexpression of Ag85 (BCG); or BCG administered in combination with IL-12 to enhance CD4+ Th1 T-cell immune responses.[41]

The worldwide epidemic of HIV/AIDS infection has had a profound impact on the epidemiology of TB and may have implications for the use of BCG.[75] In the process of designing effective and safer TB vaccine candidates it is fundamental to consider the use of these possible vaccines in a setting where HIV/AIDS infection is highly prevalent or where co-infection between TB and HIV is frequent.[41,60,62] Whole-cell inactivated vaccines such as those containing *Mycobacterium vaccae* have shown protection against TB in animal models by evoking potent mycobacteria-specific cytotoxic responses.[76–78] Human studies with this vaccine preparation have demonstrated a safety profile, as well as immunogenicity in both healthy and HIV-infected individuals.[41,76,77] However, a heat-killed *M. vaccae* vaccine demonstrated no significant protection to reduce TB in HIV-infected populations in Tanzania.[76–79]

Clinical trials will be initiated with two other whole cell vaccine candidates: one using a BCG strain that produces a similar antigen 85A, and the second one that consists of two other antigenic proteins delivered in an adjuvant formulation. A clinical phase 1 trial is underway in approximately 50 adults in the Boland–Overberg region of South Africa to evaluate the safety and immunogenicity over a 5-year period of the rBCG30 vaccine administered intradermally.[13,41] This enhanced BCG vaccine (rBCG30) was chosen for its ability to over-express the antigen 85B, when a plasmid coding for the 30-kDa protein is introduced.[13,41] The antigen 85B is a major secretory or extracellular protein and has been shown to be highly immunogenic and able to induce protective responses in mice and guinea pigs.[61,62,74]

In summary, a tuberculosis vaccine that would be able to induce protection in populations that have not had natural exposure to *M. tuberculosis* (pre-exposure) and in those that have already been exposed or that have previously received BCG vaccination (post-exposure) would be ideal.[13,14,31] The working group on vaccine development of the Stop TB initiative of the WHO has identified three major challenges to promote synergy and to accelerate identification and introduction of the most effective vaccination strategy. These challenges include initiation of phase 1 trials, developing an iterative structure for clinical trials, and the logistic and financial challenges associated with phase 3 trials.[13]

## Combined strategies to control TB in the new millennium

In the case of TB, effective global control will require the use of combined strategies. Strengthening early diagnosis of cases with institution of effective chemotherapy using DOTS is critical, however there is still a clear need for TB vaccines. Pre-exposure vaccines will be required to prevent infection and post-exposure vaccines, aimed to prevent or reduce progression to disease, will also be of benefit once a person is already infected. The protective effect of BCG demonstrated in childhood tuberculosis might be enhanced by vaccination strategies that improve BCG rather than completely replacing its use. Boosting vaccinations such as demonstrated with MVA85A could offer an efficient strategy for enhancing and prolonging anti-mycobacterial immunity in areas with elevated rates of TB.[80] It has been suggested in mathematical models

that a vaccine that can induce protection by reducing infection and preventing progression of disease might be ideal.

It is anticipated that many more new TB vaccine candidates will be developed and become available to be tested in human populations in the coming years. With the advent of improved TB vaccines, current BCG vaccination policies will be modified. However, until this moment arrives, and taking into account current scientific evidence of the protective effects of BCG vaccination, we strongly believe that in resource-limited countries, such as most developing countries, with high rates of TB infection and disease, there are compelling reasons to continue its routine use.

Finally, a major commitment from the developed world is needed to drastically improve social and economic conditions in resource-poor settings. Many have argued that without changing these structural factors, strategies such as widespread implementation of DOTS and preventative measures such as widespread vaccination with BCG, or the use of newer TB vaccine candidates will not succeed as expected in controlling the resurgent tuberculosis pandemic.

## References


1. Folch E, Hernandez I, Barragan M, Franco-Paredes C. Infectious disease, non-zero sum thinking and the developing world. *Am J Med Sci* 2003;**326**:66—72.
2. Franco-Paredes C, Agudelo C. The ever expanding clinical spectrum of tuberculosis. *J Clin Rheumatol* 2002;**8**:1—2.
3. World Health Organization. Issues relating to the use of BCG in immunization programmes. WHO 1999.
4. WHO Global Tuberculosis Programme and Global Programme on Vaccines. Statement on BCG revaccination for the prevention of tuberculosis. *WHO Wkly Epidemiol Rec*1995;**70**: 229—31.
5. Kaufmann SHE. Is the development of a new tuberculosis vaccine possible? *Nat Med* 2000;**6**:955—60.
6. Wittes RC. Immunology of bacille Calmette—Guérin and related topics. *Clin Infect Dis* 2000;**31**(Suppl 3):S59—63.
7. Iseman M. Vaccination to prevent tuberculosis and immunomodulatory therapy for persons with active disease. In: Iseman M, editor. *A clinician's guide to tuberculosis*. Philadelphia PA: Lippincott Williams &Wilkins; 2000. p. 399—419.
8. Colditz GA, Berkey CS, Mosteller F, Brewer TF, Wilson ME, Burdick E, et al. The efficacy of bacillus Calmette—Guérin vaccination of newborns and infants in the prevention of tuberculosis: meta-analysis of the published literature. *Pediatrics* 1995;**96**:29—35.
9. Rodrigues LC, Diwan VK, Wheeler JG. Protective effect of BCG against tuberculous meningitis and military tuberculosis: a meta-analysis. *Int J Epidemiol* 1993;**22**:1154—8.
10. Townsend J, Aronson J, Saylor R. Tuberculosis control among North American Indians. *Am Rev Tuberc* 1942;**45**:41—52.
11. Brewer TF. Preventing tuberculosis with bacillus Calmette—Guérin Vaccine: a meta-analysis of the literature. *Clin Infect Dis* 2000;**31**(Suppl 3):S64—7.
12. Shapiro C, Cook N, Evans D, Willett W, Fajardo I, Kock-Wesser D, et al. A case control study of BCG and childhood tuberculosis in Cali, Colombia. *Int J Epidemiol* 1985;**14**:441—6.
13. Fruth U, Young D. Prospects for new TB vaccines: Stop TB working group on TB vaccine development. *Int J Tuberc Lung Dis* 2004;**8**:151—5.
14. Ziv E, Daley CL, Blower S. Potential public health impact of new tuberculosis vaccines. *Emerg Infect Dis* 2004;**10**:1529—35.
15. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, et al. Efficacy of BCG vaccine in the prevention of tuberculosis: meta-analysis of the published literature. *JAMA* 1994;**271**:698—702.
16. Clemens JD, Chuong JH, Feinstein AR. The BCG controversy: a methodological and statistical reappraisal. *JAMA* 1983; **249**:2362—9.
17. Aronson JD. Protective vaccination against tuberculosis with special reference to BCG vaccination. *Am Rev Tuberc* 1948; **58**:255—81.
18. Ferguson RG, Simes AB. BCG vaccination of infant Indians in Saskatchewan. *Tubercle* 1949;**30**:5—11.
19. Rosenthal SR, Loewinsohn E, Graham ML, Liveright D, Thorne MG, Johnson V. BCG vaccination in tuberculous households. *Am Rev Respir Dis* 1960;**84**:690—704.
20. Rosenthal SR, Loewinsohn E, Graham ML, Liveright D, Thorne G, Johnson V. BCG vaccination against tuberculosis in Chicago: a twenty-year study statistically analyzed. *Pediatrics* 1961:622—41.
21. ten Dam HG, Pio A. Pathogenesis of tuberculosis and effectiveness of BCG vaccination. *Tubercle* 1982;**63**:225—33.
22. Tripathy SP. Fifteen-year follow-up of the Indian BCG prevention trial. *Bull Int Union Tuberc Lung Dis* 1987;**62**:69—72.
23. Fine PE. Variation in protection by BCG: implications of and for heterologous immunity. *Lancet* 1995;**346**:1339—45.
24. Talbot EA, Perkins MD, Silva SF, Frothingham R. Disseminated bacille Calmette—Guérin immunization in normal healthy adults. *J Infect Dis* 1994;**170**:476—9.
25. Puliyel JM, Hughes A, Chiswick M, Mughal MZ. Adverse local reactions from accidental BCG overdose in infants. *BMJ* 1996;**313**:528—9.
26. Turnbull FM, McIntyre PB, Achat HM, Wang H, Stapledon R, Gold M, et al. National study of adverse reactions after vaccination with bacille Calmette—Guérin. *Clin Infect Dis* 2002;**34**:447—53.
27. Kröger L, Korppi M, Brander E, Kroger H, Wasz-Hockert O, Backman A, et al. Osteitis caused by bacille Calmette—Guérin vaccination: a retrospective analysis of 222 cases. *J Infect Dis* 1995;**172**:574—6.
28. McShane H, Brookes R, Gilbert SC, Hill AV. Enhanced immunogenicity of CD4+ T-cell responses and protective efficacy of a DNA-modified vaccinia virus Ankara prime-boost vaccination regimen for murine tuberculosis. *Infect Immun* 2001; **69**:681—6.
29. Dye C. A booster for tuberculosis vaccines. *JAMA* 2004; **291**:2127—8.
30. World Health Organization. BCG Vaccine. *Wkly Epidemiol Rec* 2004;**79**:27—38.
31. Blower SM, Small PM, Hopewell PC. Control strategies for tuberculosis epidemics: new models for old problems. *Science* 1996;**273**:497—500.
32. Kaufmann SH. How can immunology contribute to the control of tuberculosis? *Nat Rev Immunol* 2001;**1**:20—30.
33. Behr MA, Wilson MA, Gill WP, Salamon H, Schoolnik GK, Rane S, et al. Comparative genomics of BCG vaccines by whole-genome DNA microarray. *Science* 1999;**284**:1520—3.
34. Tripathy SP. The case for BCG. *Ann Nat Acad Med Sci* 1983;**19**: 11—21.

35. Brewer MA, Edwards KM, Palmer PS, Hinson HP. Bacille Calmette—Guérin immunization in normal healthy adults. *J Infect Dis* 1994;**170**:476—9.
36. Sakula A. BCG: who were Calmette and Guérin? *Thorax* 1983;**38**:806—12.
37. Young DB, Stewart GR. Tuberculosis vaccines. *Br Med Bull* 2002;**62**:73—86.
38. Pym AS, Brodin P, Majlessi L, Brosch R, Demangel C, Williams A, et al. Recombinant BCG exporting ESAT-6 confers enhanced protection against tuberculosis. *Nat Med* 2003;**9**:533—9.
39. Heimbeck J. Tuberculosis in hospital nurses. *Tubercle* 1936; **18**:97—9.
40. Luelmo F. BCG vaccination. *Am Rev Resp Dis* 1982;**125** (Suppl):S70—2.
41. Fordham von Reyn C, Vuola JM. New vaccines for the prevention of tuberculosis. *Clin Infect Dis* 2002;**35**:465—74.
42. Bloom BR, Murray CL. Tuberculosis: commentary of a reemergent killer. *Science* 1992;**257**:1055—69.
43. Haverkamp MH, Arend SM, Lindeboom JA, Hartwig NG, van Dissel JT. Nontuberculous mycobacterial infection in children: a 2-year prospective surveillance study in the Netherlands. *Clin Infect Dis* 2004;**39**:450—6.
44. Valadas E. Nontuberculous mycobacteria: clinical importance and relevance to bacille Calmette—Guérin vaccination. *Clin Infect Dis* 2004;**39**:457—8.
45. Katila ML, Brander E, Backman A. Neonatal BCG vaccination and mycobacterial cervical adenitis in childhood. *Tubercle* 1987;**68**:291—6.
46. Smith D, Wiegeshaus E, Balasubranamian V. Animal models in experimental tuberculosis. *Clin Infect Dis* 2000;**31**(Suppl 3):S68—70.
47. Young CB. Building a better tuberculosis vaccine. *Nat Med* 2003;**9**:503—4.
48. Stanley SJ, Howland C, Stone MM, Sutherland I. BCG vaccination of children against leprosy in Uganda: final results. *J Hyg (Camb)* 1981;**87**:235—48.
49. Ponnighaus JM, Fine PE, Sterne JA, Wilson RJ, Msosa E, Gruer PJ, et al. Efficacy of BCG against leprosy and tuberculosis in Northern Malawi. *Lancet* 1992;**339**:636—9.
50. Karonga Prevention Trial Group. Randomized controlled trial of single BCG, repeated BCG, or combined BCG and killed *Mycobacterium leprae* vaccine for prevention of leprosy and tuberculosis in Malawi. Lancet 1996;**348**:17—24.
51. Orege PA, Fine PEM, Lucas SB, Obura M, Okelo C, Okulu P. Case control study of BCG vaccination as a risk factor for leprosy and tuberculosis in Western Kenya. *Int J Lepr* 1993;**61**:542—9.
52. Fine PEM. Primary prevention of leprosy. *Int J Lepr Other Mycobact Dis* 1996;**64**(Suppl):S44—9.
53. Leprosy Prevention Trial Madras (Abstract). BCG prophylaxis for leprosy in South India. *Int J Lepr Other Mycobact Dis* 1998;**66** (Suppl):99A.
54. Smith PG, Revill WD, Lukwago E, Rykushin YP. The protective effect of BCG against *Mycobacterium ulcerans* disease: a controlled trial in an endemic area of Uganda. *Trans R Soc Trop Med Hyg* 1976;**70**:449—57.
55. Trnka L, Pankova D, Svandova E. Six years' experience with the discontinuation of BCG vaccination. 4. Protective effect of BCG vaccination against *Mycobacterium avium intracellulare* complex. *Tuber Lung Disease* 1994;**75**:348—52.
56. Menzies D. What does tuberculin reactivity after bacille Calmette—Guérin vaccination tell us? *Clin Infect Dis* 2000; **31**(Suppl 3):S71—4.
57. Tissot F, Zanetti G, Francioli P, Zellweger JP, Zysset F. Influence of bacille Calmette—Guérin vaccination on size of tuberculin skin test reaction: to what size? *Clin Infect Dis* 2005;**40**:211—7.
58. Porco TC, Blower SM. Quantifying the intrinsic transmission dynamics of tuberculosis. *Theor Popul Biol* 1998;**76**:624—36.
59. Aronson NE, Santosham M, Comstock GW, Howard RS, Moulton LH, Rhoades ER, et al. Long-term efficacy of BCG vaccine in American Indians and Alaska Natives: a 60-year follow-up study. *JAMA* 2004;**291**:2086—91.
60. Letvin NL, Bloom BR, Hoffman SL. Prospects for vaccines to protect against AIDS, tuberculosis, and malaria. *JAMA* 2001; **285**:606—11.
61. Reed SG, Alderson MR, Dalemans W, Lobet Y, Skeiky YA. Prospects for a better vaccine against tuberculosis. *Tuberculosis (Edinb)* 2003;**83**:213—9.
62. Doherty MT, Andersen P. Tuberculosis and vaccine development. *Curr Opin Pulm Med* 2002;**8**:183—7.
63. Waldrop SL, Davis KA, Maino VC, Picker LJ. Normal human CD4+ memory T cells display broad heterogeneity in their activation threshold for cytokine synthesis. *J Immunol* 1998; **161**:5284—95.
64. Hanekom WA, Hughes J, Mavinkurve M, Mendillo M, Watkins M, Gamielden H, et al. Novel application of whole blood intracellular cytokine detection assay to quantitate specific T-cell frequency in field studies. *J Immunol Methods* 2004; **291**:185—95.
65. Kampmann B, Tena GN, Mzazi S, Eley B, Young DB, Levin M. Novel human in vitro system for evaluating antimycobacterial vaccines. *Infect Immun* 2004;**72**:6401—7.
66. Abou-Zeid C, Gares M-P, Inwald J, Janssen R, Zhang Y, Young DB, et al. Induction of a type 1 immune response to a recombinant antigen from *Mycobacterium tuberculosis* expressed in *Mycobacterium vaccae*. *Infect Immun* 1997; **65**:1856—62.
67. McShane H, Hill A. Prime-boost immunization strategies for tuberculosis. *Microbes Infect* 2005;**7**:962—7.
68. McShane HM, Pathan AA, Sander CR, Keating SM, Gilbert SC, Huygen K, et al. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. *Nature Med* 2004;**10**:1240—4.
69. Olsen AW, Pinxteren LA, Okkels LM, Rasmussen PB, Andersen P. Protection of mice with a tuberculous subunit vaccine based on a fusion protein of Ag85B and ESAT-6. *Infect Immun* 2001;**69**:2773—8.
70. Gordon A. Vaccines and vaccination. *N Engl J Med* 2001: 1042—53.
71. Tascon RE, Colston MJ, Ragno S, Stavropoulos E, Gregory D, Lowrie DB. Vaccination against tuberculosis by DNA injection. *Nat Med* 1996;**2**:888—92.
72. Turner OC, Roberts AD, Frank AA, Phalen SW, McMurray DM, Content J, et al. Lack of protection in mice with necrotizing bronchointerstitial pneumonia with bronchiolitis in guinea pigs immunized with vaccines directed against the hsp60 molecule of *Mycobacterium tuberculosis*. *Infect Immun* 2000;**68**:3674—9.
73. Huygen K, Content J, Denis O, Montgomery DL, Yawman AM, Deck RR, et al. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine. *Nat Med* 1996;**2**:893—8.
74. Horwitz MA, Harth G, Dillon BJ, Maslesa-Galic S. Recombinant bacillus Calmette—Guérin (BCG) vaccines expressing the *Mycobacterium tuberculosis* 30-kDA major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. *Proc Natl Acad Sci USA* 2000;**97**:13853—8.
75. Black GF, Weir RE, Floyd S, Bliss L, Warndorff DK, Crampin AC, et al. BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomized controlled studies. *Lancet* 2002;**359**:1393—401.

76. Skinner MA, Yuan S, Prestidge R, Chuk D, Watson JD, Tan PL. Immunization with heat-killed *Mycobacterium vaccae* stimulates CD8+ cytotoxic Tcells specific for macrophages infected with *Mycobacterium tuberculosis*. *Infect Immun* 1997; **65**:4525—30.
77. Waddell RD, Chintu C, Lein D, Zumla A, Karagas MR, Baboo KS, et al. Safety and immunogenicity of a five-dose series of inactivated *Mycobacterium vaccae* vaccination for the prevention of HIV-associated tuberculosis. *Clin Infect Dis* 2000;**30**(Suppl 3):S309—15.
78. von Reyn CF, Vuola J, Ristola M, Cole B. Immunogenicity of a multiple dose series of an inactivated vaccine for the prevention of tuberculosis in HIV infection: a blinded controlled trial. *Int J Tuberc Lung Dis* 2001;**5**:S11.
79. Sachs JD. Achieving the millennium development goals — the case of malaria. *N Engl J Med* 2005;**352**:115—7.
80. McShane H, Pathan AA, Sander CR, Goonetilleke NP, Fletcher HA, Hill AV. Boosting BCG with MV A85A: the first candidate subunit vaccine for tuberculosis in clinical trials. *Tuberculosis (Edinb)* 2005;**85**:47—52.